Chemical composition of subcritical water extraction extract of Tribulus Terrestris

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Subcritical water extraction of Tribulus terrestris leaves and fruits was performed at 100 ºC and 60 atm for 30 min. Chemical composition of volatile extracts was investigated. A simple lactone compound Loliolide (23,82%) was main components associated with 26 identified molecules which are mostly oxygenated compounds.

Introduction

In human health, plants have an important role due to certain chemicals produced by them as waste material during their metabolism.

Tribulus terrestris is one of commonly used in folk medicine in Turkey. It's mostly used due to biological activities such as diuretic, against colicky pains, hypertension and hypercholesterolemia. It is also known to increase the free serum testosterone, and then dysfunction. It has also a protective effect on genetic damage and stimulates melanocyte proliferation in the treatment of vitiligo. Several researchers were reported that *Tribulus terrestris* contains alkaloids, steroids, flavonoids and carbohydrates [1-11].

Subcritical water extraction (SCWE) is a pro-environmental process as an alternative to conventional extraction methods like solvent extraction. Under SCWE conditions, the dielectric properties of water change. The dielectric constant of water is a measure of the separation of ions from each other in other words its polarity. Under subcritical conditions, the reduced dielectric constant of water increases its dissolution properties, and therefore, it results in non-polar substances being more easily soluble. This simple method which provides high efficiency, saving time and energy, has been widely used extraction and fractionation of compounds to be obtained with a greatest specificity. Subcritical water extraction is increasingly used to extract value-added products from plant materials [12].

The aim of this study was to explore quantity of loliolide a most common mono terpenoid hydroxyl lactone extracted from Tribulus terrestris by using SCWE process which providing appropriate subcritical conditions.

Experimental part

Material and Methods

Plants material

Dried *Tribulus terrestris* was purchased from local dealer in Adana, Turkey, and was used as received.

Sub-critical water extraction (SbCWE)

Sub-critical water extraction was carried out in a laboratory built apparatuses. To remove dissolved oxygen the water was sonicated then was used in an HPLC pump. Experiments were performed at a constant flow rate, 1 $mL.min^{-1}$. The extractor heated in a temperature programmable oven. To equilibrate the water, a 2 m long pre-heated coil was used at desired temperature. The capacity of the extraction cell was 14 ml and equipped with 10 µm frit at the inlet and outlet. The extraction cell was connected to a 1m cooling loop outside of the oven. 2 g of *Tribulus terrestris* leaves and fruits were used. Extraction was carried out at 100 ˚C temperature, and 60 atm pressure for 30 min.

After a certain time, extract was collected in dichloromethane (DCM), and followed by a liquid-liquid extraction step to separate the essential oil of *Tribulus terrestris*.

GC–MS analysis

Qualification and quantification of essential oil of SbCWE extract was carried out by using a Finnigan-Trace GC–MS equipped with an auto sampler. 1 µL of sample was injected using split method with 50 split ratio. Chromatographic separations were performed with a Zebron ZB-5 capillary column (5% phenyl–95% dimethylpolysiloxane, 0.25mm i.d.×60 m, film thickness 0.25 µm). Helium was used a carrier gas with , flow rate 1.0 $mL.min^{-1}$ The column temperature was programmed from 70 to 240 ºC at $3ºC.min.^{-1}$ and the temperature of the injection port was 250 ◦C. The ionization voltage applied was 70 eV, mass range *m*/*z* 41–400 a.m.u. The separated components were identified tentatively by matching with GC–MS results of National Institute of Standards and Technology (NIST) mass spectral library data because their reference reagent were not available. The quantitative determination was carried out based on peak area integration.

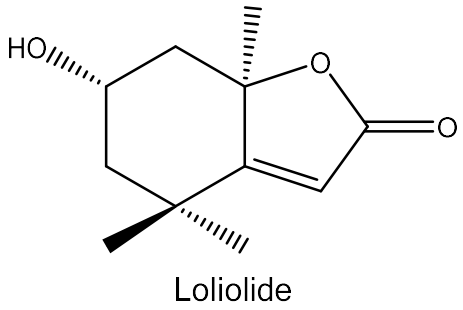
Results and discussion

To determine the chemical composition of volatile fraction of *Tribulus terrestris* extract, GC-MS analysis was employed (**Table 1**).

Loliolide which is a most common mono terpenoid hydroxyl lactone was the most abundant volatile compound in SbCWE extract of *Tribulus terrestris* (23.8 %). Despite a simple structure (**Figure 1**), *Loliolide* has a wide

spectrum of biological activity such as astringent, antipyretic, anti-inflamatory and vasodilatory effects [11]. Apart from *Loliolide*, a derivative *2-4(H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-tri methyl* was the second most abundant component of extract of *Tribulus terrestris* . The other main components of volatile compound of the extract are *coniferol* (7.9 %), *6-aza thymine* (7.8 %) *vanilline* (4.4 %), *Ethylmethylmaleimide* (3.8 %), *p-eugenol* (3.0 %), *carvone* (3.1 %), *carvacrol* (2.4 %), *p-vinyl guaiacol* (1.7 %), *Syringaldehyde*(1.7 %) and *methyl eugenol* (1.6 %). The relative amount of other detected and identified compounds was lower than 1 %. It's clearly seen that almost all the flavour compounds which were extracted from *Tribulus terrestris* via SbCWE, are oxygenated mono terpenes, oxygenated aromatic propenes or phenolic mono terpenes. These results important because subcritical water helps to isolate preferently valuable oxyganeted mono terpens from *Tribulus terrestris* [13-16].

During the SbCWE, it's possible to see degradation products such as short chain alcohols, aldehydes, aromatic and aliphatic compounds. However, we didn't observe any of these molecules in the GC-MS spectra. Hence, it can be suggest that shown that subcritical water extraction of *Tribulus terrestris* at 100 ºC and 60 atm is a suitable extraction method for the isolation of valuable components.



Loliolide

Figure 1. Structure of *Loliolide*

Conclusions

The results showed that SbCWE is a suitable extraction method to recover the important components of *Tribulus terrestris*. *Loliolide* and its derivatives such as *2-4(H)-Benzo furanone, 5,6,7,7a-tetrahydro-4,4,7a-tri methyl* are mostly presented in small quantities in plants and animals. Therefore extraction and purification of *Loliolide* is important and labor-intensive. Since, SbCWE gave a good isolation for *Loliolide* and *2-4(H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-tri methyl,* to enhance the amount of extracted *Loliolide* from *Tribulus terrestris* new parameters should be tried.

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Table 1. Essential oil components of *Tribulus terrestris* leaves and fruits were obtained by SbCWE method and they were analyzed by GC-MS

RT(min)	Compounds	Relative amount %	
		SbcWE	Brewing
11,74	Camphor	2,90	
15,32	Ethylmethylmaleimide	3,84	
15,59	**inosine**	**-**	**21,61**
15,80	Carvone	3,11	
16,96	Nonanoic acid	0,19	
18,70	p-Vinylguaiacol	1,68	
20,56	p-Eugenol	2,99	
22,19	vanilline	4,41	
22,56	Methyleugenol	1,55	
22,94	**n-formylmorpholine**	**-**	**78,38**
25,50	Veratral	0,38	
25,72	Acetovanillone	0,85	
27,42	**2(4H)-Benzofuranone, 5,6,7, 7a-tetrahydro-4,4,7a trimethyl**	**12,94**	
29,24	**6-azathymine**	**7,81**	
31,39	Elemicine	0,53	
31,56	4-fluoro-1,2-xylene	1,98	
31,67	Acetophenone	0,31	
31,84	3-Oxo-α-ionol	1,08	
31,96	verbanone	0,98	
32,40	Syringaldehyde	1,64	
32,54	carvacrol	2,58	
32,77	4-Oxo-α-ionone	0,73	
33,43	β-Ionone epoxide	2,93	
33,58	Hidroxy-β-ionone	3,25	
35,41	**Coniferol**	**7,88**	
36,89	**Loliolide**	**23,82**	
38,01	Phenol, 4-methoxy-, acetate	3,22	
38,65	Indole-3-aldehyde	1,60	
47,66	Scopoletin	0,51	

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